Reprinted from THE JOURNAL OF COMPARATIVE NEUROLOGY
Vol. 93, No. 2, October 1950

MYOTYPIC SPECIFICITY IN TELEOST MOTONEURONS

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ONE FIGURE

In man and other mammals regeneration of a severed nerve, owing to haphazard intermixing of fibers in the nerve scar and the consequent shuffling of nerve-muscle connections, leads generally to undifferentiated mass contraction and contracture of the muscles involved (Sperry, '45b). By contrast, excellent muscular coordination is recovered in amphibian limbs prior to metamorphosis even when the severed nerves are deliberately crossed into foreign muscles (Weiss, '36, '41).

The explanation, as evident from the experiments of Weiss, lies in the fact that each muscle imposes its own specificity upon the nerves that grow into it and this specificity in turn determines the temporal pattern of motoneuron discharge in the spinal centers. In amphibian larvae the limb motoneurons are readily respecified when they regenerate into a foreign muscle and the central associations are able to undergo the required readjustment. This capacity for motor reorganization persists through metamorphosis in toads but is lost shortly thereafter (Weiss, '36). It disappears much earlier in the motor ocular system of both urodele and anuran amphibians (Sperry, '47). No evidence of such reorganization has yet been obtained in mammals. The limb innervation of the rat has been found to be already beyond readjustment as early as 10 days after birth (Sperry, '41).

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To what extent the myotypic specificity and motor plasticity observed in amphibians may exist in other vertebrates and whether its presence is correlated primarily with ontogenetic or with phylogenetic variables have yet to be determined. Further information is needed particularly in view of the significant role recently ascribed to neuronal specificity in the developmental organization of the nervous system (Weiss, '36; Sperry, '50a).

MATERIALS AND METHODS

The plan of the experiments was to disarrange the normal innervation pattern of the pectoral fin musculature by breaking the nerves roughly and allowing them to undergo a haphazard regeneration into the denervated muscles. Under such conditions the individual fibers become intertangled in the nerve scar and re-establish their end-organ connections largely at random. In the absence of re-education — of which all vertebrates including man are apparently incapable under these conditions (Sperry, '45b) — it would be impossible for normal muscle coordination to be restored unless some kind of specificity were present by which the regenerated fibers could be properly distinguished from one another.

After preliminary examinations and trial operations in several species of teleosts, the southern swellfish, *Sphaeroides spengleri* (Bloch), was selected. This fish makes relatively little use of body and tail-fin movements for propulsion, relying mainly on the undulating beat of the other fins. Accordingly, the musculature of the other fins, including that of the pectorals, is highly developed and its coordination is critical to the fish and is comparatively easy to analyze. Also this species is readily obtained in numbers and is quite hardy with respect to surgery and laboratory maintenance. The animals were gathered during February and were kept individually in aquaria approximately 55 by 35 by 30 cm supplied with running sea water.

Three main muscle groups act directly upon the rays of the pectoral fin. Two of these, the adductors and abductors

of the fin, are directly antagonistic in function. They are longitudinally aligned mediad and laterad to the resting plane of the fin, and their main action is to pull the fin forward (abduction) and backward (adduction). Another somewhat smaller muscle inserted medially and at an angle to the adductors tends to elevate, spread, and adduct the fin (fig. 1).

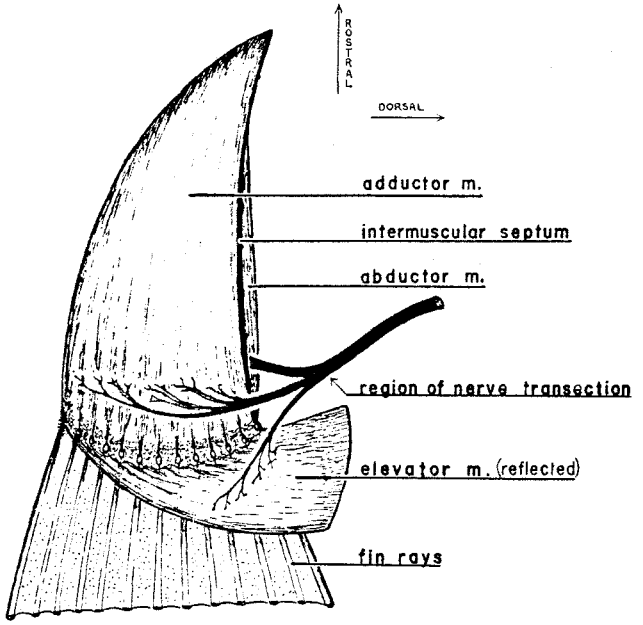

Fig. 1 Simplified diagram of the innervation of the pectoral fin muscles of *S. spengleri* (medial view).

Each of these three main muscle groups is subdivided functionally into approximately 13 parts that act through separate tendon slips upon the individual fin rays. The normal action of the fin (Breder, '26; Breder and Edgerton, '42; Harris, '36) is based on the back and forth oscillation of the

individual fin rays. A phase difference between successive beats of the rays gives a pronounced undulatory motion to the fin membrane as a whole. Differential effects are obtained by altering the amplitude, length, and speed and direction of propagation of the waves; and also by altering the posture and spread of the fin with reference to the direction of its beat, and by shifting the general posture of the fin with respect to the body.

Thus normal coordination of the fin musculature depends not only upon the differential action of each of the main muscles as a unit but also upon differential excitation of their functional subdivisions. All three muscle groups are supplied by a single large nerve trunk that arises from the most anterior spinal segments and takes a retroperitoneal course lateral and ventralward toward the base of the fin.

The nerve to the muscles of the right pectoral fin was transected in a series of 12 adult fish that ranged in standard length from 6.9 to 8.5 cm. The fin movements were noted at daily intervals thereafter while the fish were being fed. With the object of forcing increased use of the regenerated nerves, all fins except the test fin and caudal fin were cut off in 5 of the fish shortly after return of function. A control case was similarly treated for comparison. After recovery had been established, more careful checks of fin coordination were made by observing the fin while the fish were partially anesthetized. With the fin movements thus slowed and particles of feather and lint suspended in the water, it was easy to follow the different directions of the current produced by the beating fins. At the end of the experiments the regeneration pattern of the nerves was checked by electrical stimulation immediately after the fish had been decerebrated.

The operation. A dorsoventral incision through the skin was made dorsal to the base of the right fin. By separating and retracting the underlying muscles, the nerve trunk was exposed where it runs deep partially enveloped by the kidney. The nerve trunk was broken roughly by pulling and teasing it apart with finely-pointed jeweler's forceps. The

transection was made in the region just proximal to where the nerve divides into branches for the separate muscles (see fig. 1). The frayed ends of the nerve were then roughly apposed and the incision was closed with a single suture. A 1.5% solution of urethane was used for anesthesia. The operations were performed out of water under a dissecting microscope.

RESULTS

Active movement was absent in the right fin of the 12 operated fish for approximately two weeks after the nerve had been transected. Slight twitching of the fin rays was first noted on the 13th to 16th day after operation in different cases. This movement gradually became stronger during the following weeks. Although the fin movement as a whole was properly timed with respect to the other fins, it was not possible during the first 10 days of recovered function while the movements were still extremely weak to discern whether the fin beats were properly directed or whether the movements of the individual rays were coordinated with reference to each other.

On the 33rd day after operation the fin coordination was checked while the fish were recovering from light anesthesia. It was apparent at this time that good muscular coordination had been restored in 10 of the 11 cases still alive. Even in the 11th case the function, though poor, was much better than could be expected had recovery proceeded in the implastic, non-selective manner characteristic of mammals.

Haphazard re-establishment of nerve-muscle connections without central readjustment should have lead to contraction en masse of the fin muscles with no differential or adaptive adjustment in their function. Actually, the recovered function was found to be highly differential and adaptive. Strong currents were produced by the beating fins and these were differentially directed in a systematic manner at various times: caudally, laterally, and rostrally with varying dorsal and ventral components according to the particular inclination of the fish at the moment. For example, when a threatening object

approached from in front, the fins assumed a more abducted posture, the currents produced were directed rostrally and the fish backed away. Conversely if the object approached from the rear, the fins were adducted and their currents were directed caudally to propel the fish forward. While the fish were at rest, or were being tilted sideward, or were raising or lowering themselves in the water, the fins beat more laterally with varying degrees of ventrodorsal and rostrocaudal components in a manner that accurately mirrored the action of the contralateral, unoperated fins.

The final observations were made at 42–45 days after nerve transection. Nine of the original 12 fish were still alive. By this time the recovered function was good in all 9 including the one case in which coordination at 33 days had still been poor. In the two best cases the function of the re-innervated fin was indistinguishable from that of the unoperated contralateral fin and those of normal controls. The fish in which the unoperated fins had been cut off did not exhibit any more rapid or more thorough recovery than did the others, as one would have expected if re-education had been responsible for the recovery. Also in two cases so tested the recovered coordination remained unimpaired after combined bilateral excision of the entire forebrain and cerebellum. Even while these fish were recovering from the anesthesia and while the trauma of the brain excisions was still acute, there was no selective effect upon the coordination of the re-innervated fin.

Normally the nerve branches to the abductor and adductor muscle groups can be separated as distinct fascicles for some distance proximal to the point at which the nerve trunk had been transected. Faradic stimulation of these two separated fascicles produces abduction of the fin in one case and adduction in the other. The distance over which the fascicles can be separated proximally varies considerably in different individuals. In 4 of the experimental cases it proved possible at the end of the experiments to divide the nerve trunk into its separate fascicles proximal to the nerve scar. When this was done and the fascicles had been sectioned as far centrally as possible, electric stimulation proximal to the nerve scar failed to yield the differential fin movement obtained normally. Instead the fin rays merely twitched back and forth in one position. When a predominant rostral or caudal movement could be obtained, it was similar upon stimulation of each fascicle.

After its bifurcation in the nerve scar a single regenerating axon might conceivably connect with muscle fibers in two separate muscles. Evidence of such branching with differential function of the two isolated portions of the motor unit has been recorded in urodele larvae (Weiss, '37). Attempts to elicit axon reflexes in the present experiments by stimulation of the distal cut end of the regenerated nerve branch leading to the adductor muscles failed in all 5 cases tested. The presence of such reflexes in small numbers could easily have been missed, however, with the low amplitudes required to control current spread at these dimensions.

The regenerated nerves distal to the scar were approximately normal in size. The re-innervated muscles also were approximately normal, or in 4 cases, just perceptibly smaller than the contralateral muscles.

DISCUSSION

The restoration of motor coordination in these fishes was clearly much better than anything ever obtained in man and other mammals under similar conditions. The random shuffling of nerve-muscle connections caused by nerve section and regeneration failed to produce any corresponding disorder in muscular coordination. Three possible explanations must be considered: (1) The nerve fibers might have re-established their motor endings in a selective manner with the original muscles. The evidence is against selective termination of this kind in all other classes of vertebrates. Furthermore, the stimulation of separate nerve fascicles proximal to the nerve scar in the present experiments indicated a haphazard re-establishment of nerve-muscles connections. (2) Regeneration of atypical peripheral terminations might have been compen-

sated by some type of functional reorganization in the centers. A dissociation and reorganization in the spinal centers at the motoneuron level would be required. This would mean a re-integration of the primitive segmental pattern of innervation which has been maintained throughout the evolution of the specialized fin musculature (Harris, '36). The fact that functional reorganization of this kind either by re-education or by automatic reflex adjustments does not occur in any of the other vertebrates studied, including man (Sperry, '50a), makes its occurrence in fishes unlikely. Moreover, combined excision of both forebrain and cerebellum produced no selective impairment of the recovered coordination. Nor was recovery faster in those cases in which additional use of the fin had been favored by removal of the other fins. (3) Finally, the regenerated axons might have been respecified by the muscles with which they connected, as occurs in larval amphibians, with consequent readjustment in the timing of their central discharge. This latter explanation appears by elimination to be the most probable. It makes a consistent interpretation for similar phenomena in both the amphibians and fishes.

In addition to the distinct specificity of the three main muscles of the fin and their nerves, one must postulate a more refined differentiation within each muscle correlated with the orderly, in seriatim, action of the individual fin rays necessary for proper undulation of the fin membrane. This need involve only a gradient of differentiation across the muscle perpendicular to the alignment of the muscle fibers. Such a degree of refinement in nerve-endorgan specificity is not extraordinary. Equal or greater refinement has been reported to exist in the retina and optic nerve (Sperry, '43; '48), in the macula of the utriculus and its nerve (Sperry, '45a), and in the integument and its innervation (Sperry and Miner, '49).

Since the teleosts used in these experiments were mature adults, their motor plasticity cannot be considered an essentially developmental property as has been supposed in the case of the amphibians. The results support the idea that the capacity for myotypic respecification of motoneurons varies

phylogenetically as well as ontogenetically among the vertebrates, decreasing with evolutionary progression. It has been suggested (Sperry, '50a, b) that neurogenesis, in the course of evolution, may have come to depend increasingly upon central self-differentiation and selective outgrowth of motoneurons with the peripheral induction of specificity by the musculature assuming decreasing importance.

How the neuronal specificity influences the discharge pattern in the spinal centers remains speculative. Physiological sensitization of the motor cells to particular modes of central excitation has been suggested as one possibility (Weiss, '36). The central connections are assumed, in this case, to be sufficiently diffuse and non-selective so that all motoneurons within a unilateral limb center are bombarded by all modes of central excitation.

Another possibility which is in accord with a chemo-affinity concept of synaptic patterning assumes that the specificity of the motoneurons determines the kind of synaptic endings which can be formed upon the motor cells. The establishment of synaptic associations is supposed to depend upon selective affinities between the motor and association neurons (Sperry, '41, '50a). Respecification of a regenerated motoneuron by a foreign muscle is assumed to cause a breakdown of the original synaptic endings and the formation of new ones from a different set of internuncials. Evidence is lacking regarding the extent to which the motor implasticity of mammals and of adult amphibians may be due to failure of the muscles to respecify their nerves and the extent to which it may be due to the failure of such respecification to bring about the required readjustment in the centers.

SUMMARY

1. The peripheral nerve trunk that innervates the several muscles acting on the pectoral fin of *Sphaeroides spengleri* was completely transected and the frayed ends were roughly apposed. Regeneration of the severed nerve resulted in a return of muscle function that first appeared two weeks after

nerve section and reached completion approximately 20 days later.

2. Although three separate muscle groups were involved, two of which are directly antagonistic and all three of which have numerous functional subdivisions that work differentially, the normal coordinated beat of the fin rays was restored in orderly fashion.

3. Selective outgrowth of the regenerating nerve fibers to their original motor endings was disfavored by the evidence as a possible explanation as was also re-adaptation of the nerve centers on any functional basis. Myotypic respecification of the regenerated nerves, such as occurs in amphibian larvae, followed by synaptic reorganization in the spinal centers on a chemo-affinity basis is considered the most probable explanation of the results.

4. The experiments indicate that the capacity for motor readaptation following misregeneration of peripheral nerves varies phylogenetically as well as ontogenetically. Motor plasticity is even more pronounced in these fishes than in the amphibians and is more evident in both of these than in the mammals.

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